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UNITED STATES DEPARTMENT OF AGRICULTURE
Agricultural Research Service
Entomology Research Division

Proceedings of the Planning and Training Conference for Insect Pathology and Biological Control

Sheraton - Elms Hotel
Excelsior Springs, Missouri
March 31 - April 3, 1964

FOR ADMINISTRATIVE USE ONLY

UNITED STATES DEPARTMENT OF AGRICULTURE
Agricultural Research Service
Entomology Research Division
Beltsville, Maryland

February 19, 1964

To: All Conference Participants

From: E. F. Knipling, Director, Entomology Research Division

Subject: Planning and Training Conference, March 31 - April 3, 1964

By now you will have received notice that the Division is holding a special planning and training conference on the subject of insect pathology and biological control. This conference will be held at the Sheraton-Elms Hotel, Excelsior Springs, Missouri, March 31 - April 3, 1964.

This meeting will be the fourth in an annual conference series organized for the research staff of the Entomology Research Division. These meetings have been valuable as planning and training conferences for personnel engaged in specific areas of research. They are held primarily for the benefit of the Division's research programs, but scientists from other agencies in the Department of Agriculture with special interest in the conference subject have been invited to attend and participate. We recognize that other research organizations, especially State Agricultural Experiment Stations and universities, have an interest in the subjects chosen for discussion; however, these conferences are organized as in-house planning and training programs to help develop maximum effectiveness and coordination in the over-all Division program. Moreover, the Division is not in a position to organize conferences that include participation by representatives of the many States and other interested organizations without formal approval and the assistance of the appropriate officials of participating agencies. The attached program has been drawn up by the Committee appointed to plan the meeting. The Committee is composed of P. B. Dowden, H. Baker, A. S. Michael, and A. M. Heimpel, Chairman.

Speakers listed on the program are requested to submit an abstract, of no more than one page, to the Program Committee by no later than March 9, 1964. This will enable the Committee to forward copies of the abstracts to all participants before the meeting. Any questions regarding your part in the program should be submitted to the Program Committee with a copy to your Branch Chief.

You will be kept up to date on matters pertaining to accommodations, etc. by the Division Administrative Office.

E. F. Knipling

Enclosure

INSECT PATHOLOGY AND BIOLOGICAL CONTROL CONFERENCE

Sheraton-Elms Hotel, Excelsior Springs, Missouri

Purpose of the conference

During the past few decades, through the efforts and contributions of specialists in biological control in various States, in other countries, and in our own Division, interest has quickened in insect pathology as one of the most promising alternative methods of insect control. The science of control of noxious insects and weeds through the introduction of beneficial insects, although firmly established many years ago, is constantly gaining impetus as our knowledge increases.

Intensive studies in these fields have yielded parasites and pathogens worthy of development as control agents. This involves intimate knowledge of the parasite or pathogen-host relationship, development of methods to produce large numbers of the host insects in order to mass produce the parasites and pathogens; finally, adequate methods to assess the effects of release of control agents in the field are of increasing urgency.

In keeping with the efforts to develop the most effective, economical and safest insect control methods possible, the Division has substantially increased research in the field of biological control during recent years. This increased attention to biological control has led to important developments in certain areas and there is a need to exchange information, consolidate impressions and seek out new directions for the continuing research programs.

With this as its purpose, it is expected that all in attendance will come prepared to take full advantage of and contribute as much as possible to this program. Every effort will be made to provide ample time and opportunity for informal discussion to facilitate exchange of ideas.

INSECT PATHOLOGY AND BIOLOGICAL CONTROL CONFERENCE

Sheraton-Elms Hotel
Excelsior Springs, Missouri

March 31 - April 3, 1964

Tuesday, March 31

8:00-9:00 AM	Welcome and Introductory Remarks	E. F. Knipling
9:00-9:15	Announcements	E. E. Norris

INTRODUCTION
A. M. Heimpel, Chairman

9:20-10:00	Introductory remarks on insect pathology	A. M. Heimpel
10:00-10:30	Coffee break	
10:30-11:00	The pathology of the beneficial insect	A. S. Michael
11:00-11:30	Control of diseases of beneficial insects	F. E. Moeller
11:30-12:00	Summation	A. M. Heimpel

Lunch

VIRUS DISEASES OF INSECTS
C. M. Ignoffo, Chairman

1:00-1:30 PM	Use of the electron microscope in insect pathology	J. R. Adams
1:30-2:00	The potential of virus control of insects	C. M. Ignoffo
2:00-2:30	Studies on the virus disease of the citrus red mite	J. E. Gilmore

BACTERIAL DISEASE AND RESISTANCE

2:30-3:00	Virus diseases of the honey bee	J. Hitchcock
3:00-3:30	Coffee break	
3:30-4:00	Studies of B. thuringiensis and a nuclear polyhedrosis virus for control of certain insects on cabbage	C. S. Creighton
4:00-4:30	The investigation of B. thuringiensis as a control agent for the gypsy moth	F. E. Lewis
4:30-5:00	Antibacterial substances in the fodder of insects	B. Maksymiuk
5:00-5:30	Summation	C. M. Ignoffo

Wednesday, April 1

MODE OF ACTION AND PROPAGATION OF BACTERIAL PATHOGENS
S. R. Dutky, Chairman

8:00-8:30 AM	The importance of connective tissue and cell cementing substances in insect pathology	Z. E. Estes
8:30-9:00	The bio-synthesis of dipicolinic acid in relation to the *Bacillus thuringiensis* fly toxin	S. J. Louloudes
9:00-9:30	The effect of *Bacillus thuringiensis* on the honey bee larva	H. Shimanuki
9:30-10:00	The effects of *Bacillus thuringiensis* on bees, *Apis mellifera* L.	G. E. Cantwell
10:00-10:30	Coffee break	
10:30-11:00	Infection of Japanese beetle larvae (*Popillia japonica*) with *Bacillus popillia* and effect on hemolymph composition	H. H. Hall
11:00-11:30	Nutritional requirements and growth of *Bacillus popilliae* in artificial culture media	R. A. Rhodes
11:30-12:00	Summation	S. R. Dutky

Lunch

PHYSIOLOGICAL STUDIES
A. S. Michael, Chairman

1:00-1:30 PM	Host parasite relation in sterol requirements and metabolism	S. R. Dutky
1:30-2:00	Physiological larval resistance to American foulbrood in the honey bee	T. R. Hoage

PROTOZOAN DISEASES OF INSECTS

2:00-2:30	Introduction of disease using host response-eliciting compounds with special reference to the boll weevil and *Mattesia* sp. (Protozoa)	R. E. McLaughlin
2:30-3:00	Investigations of natural and applied control of grasshoppers with *Nosema locusti*	J. E. Henry
3:00-3:30	Coffee break	
3:30-4:00	The genus *Thelohania* (Microsporidia) in western mosquito larvae	H. C. Chapman
4:00-4:30	Microsporidiosis of larval Tabanidae	R. E. Gingrich
4:30-5:00	Summation	A. S. Michael

WEDNESDAY EVENING - Banquet. Two films will be shown. (1) The Biological Control Laboratory at Darmstadt, Germany. J. Franz. (2) The Control of *Neodiprion swainei* through application of specific virus. W. Smirnoff.

Thursday, April 2

THEORETICAL ASPECTS OF BIOLOGICAL CONTROL
W. H. Anderson, Chairman

8:00-8:30 AM	Importance of taxonomic investigations to biological control activities	K. V. Krombein
8:30-9:00	Experimental design and bio-control	Judson McGuire
9:00-9:30	The analysis of biological control effects through mathematical models	W. E. Waters
9:30-10:00	Logical implications of population theory	F. R. Lawson
10:00-10:30	Coffee break	
10:30-11:00	Insect population management through integrated control	E. S. Raun
11:00-11:30	Investigation potentials in bio-control	H. W. Prescott
11:30-12:00	Summation	W. H. Anderson

Lunch

PRACTICAL ASPECTS OF BIOLOGICAL CONTROL
G. J. Haeussler, Chairman

1:00-1:30 PM	Reproductive capacities as indicators of the effectiveness of three parasites of *Therioaphis maculata* in various climates	D. C. Force
1:30-2:00	Specificity in host-parasite relationships with particular reference to parasites of *Hypera postica* and their relationship to other species of the genus *Hypera*	Benj. Puttler
2:00-2:30	Biological control of *Cephus* in Eastern United States - A success or failure	F. A. Streams
2:30-3:00	Density dependent factors versus density independent factors as biological control agents	A. G. Selhime
3:00-3:30	Coffee break	
3:30-4:00	Parasites and predators of aphids in Maine	W. A. Shands
4:00-4:30	Methods used in propagation, colonization, and recovery of some introduced parasites of the alfalfa weevil	Leon Coles
4:30-5:00	Two films will be shown. (1) Biological control at Riverside, California. (2) On biological control in Australia.	

Friday, April 3

BIOLOGICAL CONTROL OF FOREST INSECTS
P. B. Dowden, Chairman

8:00-8:30 AM	Introduction of *Agathis pumils* against larch casebearer in Idaho	D. E. Parker
8:30-9:00	Biological control of forest insects in the Southeastern Region with special emphasis on the balsam woolly aphid	R. J. Kowal
9:00-9:30	Studies of diapause in several species of parasitic insects	R. B. Ryan

BIOLOGICAL CONTROL OF WEEDS

9:30-10:00	Current domestic research in the biological control of weeds	R. B. Hawkes
10:00-10:30	Coffee break	
10:30-11:00	Mechanisms of host acceptance in weed feeding insects	D. C. Force
11:00-11:30	Biological control of aquatic weeds	W. H. Anderson
11:30-12:00	P. L. 480 projects and their value to biological control programs in the United States	P. B. Dowden

Introductory Remarks on Insect Pathology

A. M. Heimpel, Entomologist
Insect Pathology Laboratory
Beltsville, Maryland

The microorganisms that attack insects number several hundred and represent most of the known microbial groups including viruses, bacteria, fungi, protozoa, and rickettsia. A general description of these microorganisms, the appearance of the infected insects and the histopathological symptoms will be discussed with liberal use of photographs.

The Pathology of the Beneficial Insect

A. S. Michael, Microbiologist
Apiculture Research Branch
Beltsville, Maryland

A general discussion, limited to the pathology of the honey bee and its importance to agriculture. The microflora of this insect will be discussed in some detail with additional emphasis on those organisms pathogenic to the honey bee. The aim of this paper will be to lay the basic groundwork for the papers that will follow and to refresh the knowledge of the conferees on the diseases of the honey bee.

Transparencies will be used to illustrate both the general and specific microflora of the honey bee and the more important infectious and noninfectious diseases of this insect.

Control of Diseases of Beneficial Insects

Floyd E. Moeller, Entomologist
Apiculture Research Branch
Madison, Wisconsin

Honey bees have two important bacterial diseases of their brood: American foulbrood caused by Bacillus larvae White, and European foulbrood caused by Streptococcus pluton (B. pluton White). B. eurydice, along with S. pluton, produces a more virulent type of EFB. B. alvei, B. laterosporus, and S. apis are secondary invaders in larvae dead of EFB. B. alvei produces an antibiotic that slows the growth of S. pluton. Spread of these diseases is by drifting bees, contaminated equipment, or pollen. Spores of AFB are highly resistant to heat and may also be spread in honey. Treatment of AFB has been by eradicating and burning infected colonies, although drugs such as sulfathiazole or terramycin in sugar sirup or powdered sugar dust are effective in prevention. Streptomycin, terramycin, or erythromycin are effective against EFB.

Adult honey bees are attacked by a protozoan Nosema apis Zander. This spore-forming protozoan attacks the lining of the midgut and shortens the life of bees by 50 percent, besides impairing the ability of nurse bees to care for brood. It is most serious under conditions where bees are confined or restricted in brood rearing. Good beekeeping practice reduces its effect, and the use of the antibiotic fumagillin fed continuously in food supplements over a period of at least 3 weeks will control it.

A mite, Acarapis woodi, which attacks adult bees through the prothoracic spiracles, has effects similar to nosema disease. This mite is found in other countries but to date is not in North America. An embargo against importation of adult bees or combs into the United States or Canada has been effective. Treatment in Europe has been by fumigation of the bees with chemical smokes.

Other adult bee diseases of lesser economic importance include: paralysis caused by a virus and associated with stock susceptibility; septicemia caused by B. apisepticus; amoeba disease caused by an amoeba attacking the midgut and Malpighian tubules; gregarines; and nematodes. Sacbrood, caused by a virus, may attack brood.

Poisoning of bees and brood from insecticides may at times be confused with diseases. Of particular note in recent years is poisoning by Sevin.

Use of the Electron Microscope in Entomology

Jean R. Adams, Entomologist
Insect Pathology Laboratory
Beltsville, Maryland

The first electron microscope was built and demonstrated in Germany in 1931 by Knoll and Ruska, two graduate students at the High Tension Laboratory of the Technical University in Berlin. With further modifications, by 1933, Ruska was able to obtain magnifications of 8 - 12,000. Since then many improvements have been made both in speed of operation and increase in resolution so that the practical resolution obtained (10 Å) and one is able to make observations at magnifications from 2,000 to 200,000.

The electron microscope has become an indispensable tool for the direct study of ultrastructure. Its value as a tool in insect pathology and insect physiology will now be considered and demonstrated.

Examination of Insect Pathogens

Although insect polyhedral viruses can be resolved with the light microscope, one is able to determine the following additional information from electron microscopic examination: (1) the type of virus, i.e., nuclear or cytoplasmic; (2) the shape, size, and number of the virus rods or particles enclosed within each developmental membrane, and (3) the structure of the protein matrix in which they are embedded. One may examine directly the small non-inclusion viruses and the virus rods of granulosis viruses. Techniques involved in a study of insect viruses include: purification procedures; chemical treatment to free virus particles from the protein matrix; shadowing with metals, preparation of carbon replicas, and negative staining to learn something of polyhedral shape and surface structure; and preparation of ultrathin sections. Some of these techniques are also applicable for the study of bacteria, protozoa and fungi.

Histopathology

The pathogenesis of a microorganism in insects is determined, in part, by histological techniques. Histological examinations can be made only on specimens that are received alive. For a proper comparison healthy specimens should be sent with diseased specimens. Whole insects are prepared for paraffin sections for light microscopy and tissues are fixed and embedded for electron microscopy. For orientation purposes a thorough light microscopic study always precedes an electron microscopic examination. A field in the light microscope on oil immersion is approximately 0.15 mm in diameter while in the electron microscope it is approximately 10 μ at 10,000x, 4 μ at 25,000x and 2 μ at 50,000x.

Insect Physiology

Insect physiologists have sought ultrastructural information so that they might better interpret data obtained in electrophysiological, biochemical, and behavioral response studies. At present our laboratory is cooperating with the Insect Physiology Laboratory and other USDA workers in projects on vision, chemoreception, and insect resistance.

Limitations in Electron Microscopy

Some problems involved in a project involving electron microscopy are summarized below: (1) The sample size observed is always small. Generalizations concerning a specific insect or groups of insects cannot be made. (2) The data obtained are not readily adaptable to statistical treatment. (3) Facts must be distinguished from the artifacts that frequently result using present techniques. (4) More time and effort is required in insect histology since the insect cuticle acts as a barrier to prevent penetration of fixatives and embedding materials. (5) Much time is required for examination of tissue sections in the electron microscope since field size is so small at high magnifications. (6) Maximum resolution is achieved through maintenance of a microscope column uncontaminated. This requires considerable time in cleaning of apertures, pole pieces, etc. (7) Positive identification of an insect pathogen can never be based on electron microscopic evidence alone; Koch's postulates must first be demonstrated.

The Potential of Virus Control of Insects

Carlo M. Ignoffo, Entomologist
Pink Bollworm Research Laboratory
Brownsville, Texas

Virus diseases of insects are potentially our most effective insect pathogens. By now more than 200 species of insects and mites are known to have virus diseases. Literature reviews present striking examples that viruses can be used to control insects. But, why aren't viruses more widely used? The major reason, indeed the most obvious, is that viruses are not available to growers. Successful experimental programs, which show that viruses can be propagated under controlled conditions, should interest and eventually stimulate commercial concerns to produce viruses and make these available to growers. The practicality of propagating viruses under controlled conditions was initially demonstrated with the nuclear polyhedrosis virus of the cabbage looper, *Trichoplusia ni* (Hübner).

Later (1963) an experimental pilot program was started to explore the possibility of propagating a nuclear polyhedrosis virus of the corn earworm, *Heliothis zea* (Boddie), and tobacco budworm, *Heliothis virescens* (Fabr.), from larvae reared on a semi-synthetic diet. The pilot program was designed to answer three basic questions: (1) Can the nuclear polyhedrosis virus be produced and in quantities large enough for field application? (2) Is the virus thus produced effective and (3) Are production techniques and costs within the realm of commercial feasibility?

The results of the bollworm pilot program will be presented and related to possible production of other fastidious insect pathogens.

Studies on the Virus Disease of the Citrus Red Mite

J. E. Gilmore, Entomologist
Fruit and Vegetable Insects Research Branch
Riverside, California

Since its discovery in 1958, a virus disease that attacks the citrus red mite (<u>Panonychus citri</u> [McGregor]) has been under investigation as a potential biological control agent of this mite. Symptoms are manifested externally by a sudden stiffening of the legs ventrad from the body, and internally by the production of characteristic birefringent crystals.

Instability of the pathogen has created certain storage problems but frozen glycerine suspensions, freeze-drying and vacuum desiccation of diseased-mite material, have proved satisfactory for accumulating and storing inoculum.

The disease can be transmitted by spray application of diseased mite suspensions and by introduction of infected mites into healthy populations. The latter method is the most efficient in transmitting disease to low density populations. Recent tests have indicated that the primary mode of transmission may be through injecting the pathogen into the plant tissue by infected mites in feeding and later being ingested by healthy mites feeding at these same or adjacent sites. Topical applications of virus suspensions to healthy mites are noninfective. Also, the disease is not transovarian.

Dried deposits of the aqueous suspensions on plant surfaces generally remain infective for only a few hours whereas natural deposits from diseased mites remain infective for several days. In preliminary tests to determine the thermal point of inactivation, aqueous suspensions withstood 6 hours at 104° but were inactivated by 2 hours at 122° and 30 minutes at 149° F. Aqueous suspensions are also inactivated by freezing and thawing. The pathogen is denatured by treatment for 30 minutes in sodium hypochlorite at concentrations of from 0.5 to 1.0 percent.

In field experiments conducted at two different locations, treatments have been made at approximately 6-week intervals by the application of virus sprays and by introduction of laboratory-inoculated mites. Populations have remained below economic levels for more than one year at both locations but the role of the disease independently of other biological and climatic factors has been difficult to assess. In a third location treatments by the introduction of different numbers of laboratory-inoculated mites have been made only when infestations were heavy. Following the introduction of 10,000 laboratory-inoculated mites per tree into a heavy infestation recently, an epizootic occurred resulting in approximately a 15-fold decline in the pretreatment population.

Virus Diseases of the Honey Bee

J. D. Hitchcock, Entomologist
Apiculture Research Branch
Laramie, Wyoming

There are several types of "paralysis" of adult honey bees, most of them caused by various poisons, but at least one type was proven infectious by Burnside (1933). He (1945) at Laramie, transmitted this paralysis by feeding, spraying, or injection of bacteria-free filtrates, whereas heat-sterilized filtrate did not produce the symptoms or mortality. No inclusion bodies, typical of most viruses affecting insects, are present. Morison (1936) in England claimed there were granular "inclusions" in the epithelial cells at the anterior end of the small intestine in paralyzed, but not in healthy, bees. However, Lotmar (1940) in Switzerland claimed these had no relationship to paralysis or a virus. Giordani (1955) in Italy claimed the ventricular epithelium is modified in one type of paralysis. Savoy (1959) in Bulgaria inactivated the virus of paralysis by heat, and claimed that biomycin fed in sugar sirup to bee colonies controlled the disease. Butler (1962) at Rothamsted reported that pathogenicity was correlated with the number of "virus particles" in the inocula, and that honey bee paralysis was transmitted experimentally to bumblebees, but not to wasps or 4 non-hymenopterous insects. An apparent case of paralysis from the State of Washington was transmitted to caged bees at Laramie.

White (1917) in the U. S. first named and carefully described sacbrood, a disease affecting honey bee larvae, and showed by experimental inoculations that it is caused by a non-inclusion virus. Its infectiousness was confirmed by Morgenthaler (1918) in Switzerland. Katznelson and Jamieson (1952) in Canada also transmitted sacbrood experimentally with bacteria-free filtrates, and stated that chloromycetin was of some value in preventing the disease. Steinhaus (1949) in the U. S., and Brcak, et al. (1963) in Czechoslovakia, have demonstrated by electron micrography spherical particles which may be virus particles in sacbrood larvae. Fyg (1956, 1959, 1962) in Switzerland believed the formation of the characteristic sac of fluid is due to secretion of excessive molting fluid, caused by the virus attacking glands affecting hormonal secretion. He produced similar symptoms by artificial ligatures and by surgical removal of the corpora allata. At Laramie, the speaker has transmitted sacbrood by contamination of the food in individual brood cells with 1 to 2 microliter droplets of various filtrates of aqueous extracts of diseased larvae. These included bacteria-free filtrates. Up to 90% of these larvae died of sacbrood, but with wide variations in the percentages in different tests. Control larvae "inoculated" with heat-sterilized filtrate or with sterile water, in alternate rows of brood cells in the same combs, showed negligible infections. There was a rapid decrease of infectivity after death of the larvae. Larvae of all ages from hatching to 5 days old were susceptible, but there was some evidence of increasing resistance with increasing age.

Studies of Bacillus thuringiensis and a Nuclear Polyhedrosis Virus for Control of Certain Insects on Cabbage

C. S. Creighton, Entomologist
Fruit and Vegetable Insects Research Branch
Charleston, South Carolina

Residues of the commercial *Bacillus thuringiensis* products Bakthane L69, Biotrol, and Thuricide on cabbage foliage outdoors did not remain pathogenic to third-instar cabbage loopers as long as residues of these materials kept indoors. Laboratory and field studies showed that these bacillus materials inhibit feeding by the cabbage looper. A high dosage of Bakthane L69 applied to small field plots of cabbage was comparable to naled and parathion in protecting cabbage plants from feeding by the looper and fall armyworm. Corn oil added to Bakthane L69 resulted in added plant protection from feeding by the cabbage looper. Bacillus formulations gave good control of the imported cabbageworm.

Residues of a cabbage looper nuclear polyhedrosis virus culture on microscope slides held indoors were more pathogenic to third-instar cabbage loopers than residues on slides held outdoors. The addition of an alkali to a suspension of the virus decreased its pathogenicity to the cabbage looper. Prolonged storage of virus suspensions in a household-type refrigerator resulted in a considerable loss of polyhedra. The virus was comparable to parathion in controlling loopers in small field plots of cabbage.

Investigation of Bacillus thuringiensis as a Control Agent for the Gypsy Moth

Franklin B. Lewis, Entomologist
Forest Insect and Disease Laboratory
New Haven, Connecticut

In 1961 a small scale field test of a Bacillus thuringiensis wettable powder formulation was conducted against the gypsy moth in New York State. The results of this test, although inconclusive, were encouraging enough to lead to a larger more comprehensive test in 1962 by the same cooperating agencies. This test was also encouraging, but inconclusive. The announcement in late 1962 of a new emulsifiable concentrate appeared to alleviate the formulation and suspendibility problems that seriously limited the 1961 and 1962 tests. In 1963 a third field test was conducted using this new concentrate. The lower dosage used in this test gave high larval kill, but did not result in practical control as determined by a pre-set number of residual egg masses per acre.

Each of the three tests presented a different set of problems which apparently blunted the full effectiveness of the material as used in the field. In 1961, the main problems were with the application of the material and the sticker and emulsifier used. In 1962, the major difficulty was poor suspendibility of the powder with the result that much less material was applied to the plots than specified. In 1963, the mixability and application problems were completely solved, but the biological activity of the material and the feeding activity of the larvae were not normal resulting in poorer results than anticipated.

The final conclusions of these three field tests of Bacillus thuringiensis against the gypsy moth are that the use of B. thuringiensis definitely is a promising method of control of this insect, but more information is needed on the nature and amount of toxin(s) required to kill. In addition, more precise information is needed on the assessment of spray coverage and deposit of microbial insecticides. And finally, it is imperative that the B. thuringiensis material to be used in field applications be thoroughly pre-tested against the target insect.

Antibacterial Substances in the Fodder of Insects

Bohdan Maksymiuk, Entomologist
Forest Insect Laboratory
Beltsville, Maryland

Antimicrobial substances have been reported from 157 families of vascular plants. They occur in leaves, stems, bark, roots, seeds, and fruits of many plants. They protect some plants from diseases caused by certain bacteria, fungi, and virus.

At the same time, these or other antimicrobial substances in plants may pose a problem in microbiological control of phytophagous insects. They might be important in determining the susceptibility of some insects to certain disease-causing organisms.

In evaluating the potential of various pathogens in forest insect control, we are emphasizing the dynamic role of the host trees in the pathogen-insect-plant complex. Substances have been found in local trees (Virginia pine, loblolly pine, pitch pine, Norway spruce, eastern hemlock, sweetgum and white oak) that are antibiotic to Bacillus thuringiensis Berliner. Twenty-seven strains of B. thuringiensis, that were tested, were inhibited by the raw extracts from pitch pine foliage.

An attempt is being made to (1) isolate and identify the antibacterial substance in pitch pine foliage, (2) to develop a strain of B. thuringiensis resistant to this substance, and (3) to determine what groups of insect pathogens are affected by antimicrobial substances in different trees.

Different solvents and chromatographic techniques are being used for purification and isolation of the active principles from pitch pine foliage. The fractions are characterized and U.V. and infra-red spectra determined.

Preliminary investigations indicate that substances in pitch pine foliage are chemically related to tannins, they are aromatic carboxylic acids with polyhydroxy functional groups. Converting the carboxyl group to a methyl ester did not destroy the antibacterial activity.

Later, a comparative study will be made of the active substances in another tree species.

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The Importance of Connective Tissue and Cell Cementing Substances in Insect Pathology

Zane E. Estes, Entomologist
Insect Pathology Laboratory
Beltsville, Maryland

The importance of cell cementing substance in insect pathology lies chiefly in its suspected ability to provide a passageway through and between body tissues for invading pathogens and their associated toxins. In the past, insect cell cementing substance has been considered to be of a mucopolysaccharide nature, on the basis of limited histochemical evidence. However, with the exception of chitin, chemical evidence of the occurrence of mucopolysaccharides in insects was lacking.

Preliminary to investigations of the definitive role of cell cementing substance in insect pathology, it therefore became necessary to extract, isolate, and identify this material. This project has now been completed and the results of it may be summarized as follows:

Midgut cell cementing substance has been isolated from the greater wax moth, Galleria mellonella (Linnaeus). The elution pattern of this material from the anion exchanger ECTEOLA cellulose showed the presence of a single component, identical in acid-base dissociation properties to mammalian hyaluronic acid. Subsequent analyses of the insect mucin showed the absence of sulfate radicals, further distinguishing it from chondroitin sulfate and heparin. Qualitative and quantitative data showed the insect cell cementing substance to be a mixed polymer of N-acetyl-glucosamine and glucuronic acid. As such, it is identical with mammalian hyaluronic acid.

The importance of these findings will be discussed as they pertain to future research plans.

The Isolation of Dipicolinic Acid and its Relationship to the *Bacillus thuringiensis* fly toxin

Spiro J. Louloudes, Entomologist
Insect Pathology Laboratory
Beltsville, Maryland

Cooperative investigations between the Beltsville Laboratories of Insect Pathology and Physiology reported the production of exotoxin by various crystal forming bacteria related to *Bacillus thuringiensis* var. *thuringiensis*.

Bioassay techniques were developed using the house fly, and the susceptibility of several species of insects to the toxin was investigated. Attempts to concentrate and isolate the toxic component were partially successful and are still in progress.

The toxic component was found to be accompanied by a strong U.V. absorbing compound. The chemical characteristic of the isolated compound was identified as the calcium salt of dipicolinic acid. When this non-toxic pyridine salt was examined in toxic and non-toxic producing strains of *B. thuringiensis*, its characteristic triple peak was found to be associated with the toxin producing varieties. In the non-toxic producing strains the U.V. spectra were similar to that of free dipicolinic acid.

Subsequent studies on improved techniques of bioassay and some of the chemical characteristics of the fly toxin will be reported. The use of C^{14}- labeled precursors their potential role as markers to assist in the elucidation of the toxic moiety (ies) and its relationship to dipicolinic acid will be discussed.

The Effect of *Bacillus thuringiensis* on the Honey Bee Larva

H. Shimanuki, Microbiologist
Apiculture Research Branch
Laramie, Wyoming

A number of studies have shown that *Bacillus thuringiensis* is innocuous to the honey bee, *Apis mellifera*. These studies for the most part were concerned with the adult bees or the entire colony without regard to the individual larva. The present study was conducted using techniques that are applied to the study of larval diseases of the honey bees.

The pathogenicity of *B. thuringiensis* to the honey bee larvae was studied using two lines of bees: one bred for its resistance to American foulbrood (AFB) and the other bred for its susceptibility to the disease. Larvae of 16-, 24-, and 32- hours of age of both lines were given one of three treatments: distilled water, spores of *Bacillus larvae* (cause of AFB), or spores and crystals of *B. thuringiensis*. The treatments were administered to individual larva by contaminating the larval food.

No evidence of disease was seen in larvae that received *B. thuringiensis*, whereas larvae that received spores of *B. larvae* developed typical symptoms of AFB. Nurse bees removed from the cells larvae that received *B. thuringiensis* at a rate higher than those receiving *B. larvae* one day after feeding. Statistically significant differences in removal rates as a result of feeding *B. thuringiensis* could not be found in the AFB resistant line larvae although apparent differences could be seen. In the susceptible line larvae, the difference in removal rates due to feeding *B. thuringiensis* after one day was significant in only the 16- and 24- hour larvae.

No attempt was made to isolate the fraction(s) which was responsible for the removal of the larvae after being fed *B. thuringiensis*.

Mortality of Honey Bees, *Apis mellifera* Linnaeus,
Fed *Bacillus thuringiensis* var. *thuringiensis* Berliner Exotoxin

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The exotoxin was removed from a commercial preparation of *Bacillus thuringiensis* and fed at different concentrations to larval and adult honey bees, *Apis mellifera*. The lowest concentration (0.312 mg per ml of sucrose solution) killed 5.4% of the larvae after 12 days with nearly 95% emerging as adults, whereas the highest concentration (20.0 mg per ml) killed all but 10.8%. No kill was recorded after 5 days when a concentration of 0.625 mg per ml was fed to adult bees, but the high concentration of 20 mg per ml killed nearly 100% in 4 days.

Infection of Japanese Beetle Larvae (*Popillia japonica*) with *Bacillus popilliae* and Effect on Hemolymph Composition

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Larvae of the Japanese beetle are infected with both vegetative cells and spores of *Bacillus popilliae* and *Bacillus lentimorbus* to (1) determine the relative degree and constancy of infectivity of strains maintained under laboratory conditions, (2) study the effect of experimental environments of cultures on infectivity, and (3) provide spores and hemolymph from diseased larvae for analysis in a search for factors that might lead to good growth and sporulation of these organisms *in vitro*. To maintain maximum viability for infectivity assays, vegetative cells are suspended in 0.1% tryptone. The number of cells injected per larva to obtain optimal infectivity is variable, and is influenced by the cell growth medium and strain of organism. Lyophilized cultures of each *Bacillus* species remain viable and infective for more than 3 years.

Amino acids in the hemolymph from healthy and diseased larvae have been determined. During the course of infection by *B. popilliae*, amounts of glycine, tyrosine and histidine decrease; amounts of glutamic acid, beta-alanine, aspartic acid and phenylalanine increase markedly, and there is some increase in threonine, serine and lysine concentrations. When larvae were infected with *B. lentimorbus*, amounts of serine, tyrosine, proline, glycine, alanine, isoleucine, histidine, arginine and valine decrease, whereas, glutamic acid increases. Acetic acid accounts for 75% of the volatile acid content of the hemolymph and there are small amounts of butyric, propionic and formic acids present. Volatile acids do not change appreciably during the course of the disease. Non-volatile acids include lactic, succinic, malic, tartaric, citric, glycolic, and gluconic acids. During the course of infection by *B. popilliae*, amounts of malic, tartaric, and glycolic acids increase. Trehalose is the major carbohydrate present in the hemolymph, which may decrease nearly 50% during the course of the disease. The oxygen content of hemolymph decreases markedly during the period of growth of vegetative cells but returns to near normal upon sporulation of the cells. Hemolymph from healthy larvae was assayed for vitamins and mineral elements. The effects of these components on the growth of the milky disease bacteria will be discussed.

Nutritional Requirements and Growth of *Bacillus popilliae* in Artificial Culture Media

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The use of the milky disease organisms as control agents for the Japanese beetle requires large numbers of infective spores which, in turn, necessitates first the production of large numbers of vegetative cells. Growth of the milky disease organisms *in vitro* is typical of bacterial growth. Cultures can be maintained by periodic transfer of viable vegetative growth or the vegetative cells can be lyophilized to provide stock cultures.

The pathogens may be grown readily in shaken flasks or in larger quantities in aerated, stirred fermentors. Rapid cell proliferation requires a comparatively rich medium of neutral pH, the presence of a fermentable carbohydrate, and oxygen. Abundant cell yields are obtained on media formulated with complex organic nitrogen sources which contain the required amino acids and growth factors. Media based on yeast extract have been used routinely with most success.

However, other media containing less expensive ingredients such as corn steep liquor and casein hydrolyzates often afford comparable yields of cells. Carbohydrates are dissimilated by *Bacillus popilliae* only aerobically via the EMP and HMP pathways to lactic and acetic acids, and CO_2 as the primary products. Alternative participation of these pathways depends upon oxygen availability and the proportion of acetic to lactic acid varies, although the total amount of acid produced is constant. Terminal oxidation of acetate can occur slowly in oxygen-enriched atmospheres. Aeration adequate to provide necessary oxygen is necessary for maximum vegetative growth and maximum oxygen demand by cultures coincides with most rapid growth. Correspondingly, the amount of dissolved oxygen in the hemolymph of diseased larvae decreases significantly during the period of vegetative growth of the pathogens. Maximum growth rates of *B. popilliae* in the laboratory consistently yield about $5x10^8$ cells per ml in 20-24 hr incubation periods. This is at least 10-20 times fewer cells than occur in the larval hemolymph as a result of the infection process.

Alternative methods of propagation including diphasic culture or procedures designed to neutralize acids or to remove products by dialysis have not markedly enhanced the yield of cells per volume of

nutrient medium, although viability may be somewhat prolonged. *In vitro*, the period of maximum cell yield characteristically is followed by death of the cultures at a rate roughly proportional to the rate of growth. The comparable growth phase in larvae is followed by rapid spore formation by as many as 90% of the vegetative cells present. As yet, no method of handling cultures in the laboratory has absolved cells from loss of viability. Cell death is not accompanied by complete dissolution of cell structure but leakage of cell contents and internal granulation is observed. The implications of these observations to spore formation will be discussed.

Host-Parasite Relation in Sterol Requirements and Metabolism

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Studies on these relationships represent the cooperative effort of my group and the Insect Physiology Laboratory. Presently under study is the DD-136 nematode and its propagation host, the larvae of the greater wax moth, Galleria mellonella (Linnaeus). The findings to date in this investigation are reported herein. Other organisms also under study include a yeast-like symbiote of the cigarette beetle and the microbial moderation of sterol requirements of house fly larvae on synthetic diets.

Examination of the infective stage nematodes propagated on the wax moth showed about 55 percent of the sterol present in the nematode was lathosterol (Δ^{7} cholestenol), and about 45 percent was cholesterol. As nearly all of the sterol in the host insect is cholesterol, further study will be directed toward establishing whether the cholesterol of the insect is converted to lathosterol, or whether the nematode produces lathosterol from other starting materials.

The rapid kill of the host by the DD-136 nematode is due to the introduction of the specifically associated pathogenic bacterium and the nematode develops in the host cadaver preserved from putrefaction by the wide spectrum antibiotic produced by the bacterium. It was important to determine whether the bacterium itself altered the lipids and sterols of the host. Larvae killed by injection of a pure culture of the associated bacterium were examined 11 days after innoculation, and no significant shift in the sterols was found. In the cadavers in which both nematodes and the bacterium had been introduced, significant amounts of lathosterol were present.

For identification of the nematode sterols, nearly 1/3 of a billion infective stage larvae were processed, the bulk of which were subjected to immediate saponification. These nematodes yielded a total mass of 255 milligrams of non-saponifiable material from the 70 grams of live weight of nematodes processed, of which 21.7 milligrams, including the sterols, were eluted in the alcohol fraction following column chromatography on alumina. By making the azoate derivatives of the alcohol fraction, two major components were isolated - 10 milligrams of lathosterol and 6 milligrams of cholesterol. Adequate amounts of both were obtained to make positive identification.

Two 3-gram samples of the nematode were used for total lipid analysis, and from these samples the quantity of sterols per nematode was estimated as 9.5×10^{-5} micrograms or 95 picagrams per nematode.

Selection of Physiological Larval Resistance to American Foulbrood in the Honey Bee

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Selection for larval resistance to American foulbrood was initiated in the F_1 generation of a susceptible line by resistant line cross. Female larvae 0 to 6 hours old and male larvae 0 to 12 hours old were treated with 1,000 AFB spores in 0.287 mm^3 of distilled water. The survivors (designated I) were mated in combination with nontreated individuals (designated N). Another class was the mating of the most susceptible individuals as determined by progeny tests. Progeny tests were run using 1,000 spores for each 18 to 24 hour old individual of the above matings. Larval weights were taken at the time of inoculation on samples of 10 larvae. These methods were followed for three generations of selection.

The first generation of selection gave a significant difference in larval mortality between mating classes. The most effective selection occurred in the N x I or gamete selected group. Interaction in selected gamete and selected zygote matings resulted in a gain of less magnitude than in either of the other two selected resistant classes. Larval weights were correlated with mortality response and had an overall correlation coefficient of -0.3000. Although not significant at the 0.05 level of probability, it appeared evident that the heavier larvae were more likely to resist an infection of AFB spores. A correlation value of -0.9671 occurred in the I x N class of matings, showing a high correlation between larval weight and resistance.

Generation two responded in the same manner but with a smaller increase in resistance. Interaction was again present in I x I matings. The overall r value of -0.4970 for the weight-mortality response was significant indicating an even higher correlation between larval weight and resistance after two generations of selection.

The third generation was tested during a dearth period. Sufficient larvae were not available for both treatments, and larval weights were excluded. Mortality responses differed greatly from the two previous generations with an increase in mortality in all but the I x I mating class. None, however, returned to the level of mortality observed in the first generation.

Egg weight and hatching time were observed to be influenced through selection for resistance.

Introduction of Disease Using Host Response-eliciting Compounds With Special Reference to the Boll Weevil and *Mattesia* sp. (Protozoa)

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Response-eliciting compounds have been detected in cotton plants and can be extracted. These substances elicit specific behavioral responses in the boll weevil, *Anthonomus grandis* Boheman, such as feeding, attraction or repellency. The substance usually referred to as a feeding stimulant or arrestant, has been used in an attempt to alter the feeding habit of the weevil, resulting in ingestion of the spores of *Mattesia* sp. Limited field tests during 1963 resulted in about 67% infection of weevils by this method. Further tests will be conducted.

Although the feeding stimulant-*Mattesia* combination was initially tested against the boll weevil, the principle involved opens a new area of biological control techniques. Host response-eliciting compounds have already been detected in other plants, and there are strong indications that these substances may be widespread in insect-host plant relationships. Employment of these materials to alter the behavior of the insect, even if only for one short period of time, could permit the use of a large number of pathogens which have previously seemed impractical. Specifically, pathogens which must be taken *per os* to infect, and which are infective to an insect species whose feeding habits make placement of the pathogen where the insect would ingest it either impossible or impractical, might become useful biological control agents.

Investigations of Natural and Applied Control of Grasshoppers with *Nosema locustae*

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Preliminary laboratory studies have shown that *Nosema locustae* Canning (Sporozoa : Microsporidia : Nosematidae) is an effective pathogen of grasshoppers. It is intracellular in fat body cells of the host, which results in increased mortality rates, reduced fecundities, and reduced sizes of grasshoppers. Under natural conditions the organism is transmitted by grasshopper fecal material, cannibalism, and by transovarial transmission.

Field studies of naturally occurring and artificially induced epizootics have been initiated to evaluate the use of this protozoan for controlling grasshoppers. Under natural conditions the disease increases in both infection levels and rates during a single season. Field applications of spores have established the disease in areas where it had not been present in grasshoppers.

The Genus *Thelohania* (Microsporidia) in Western Mosquito Larvae

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Prior to 1960, no *Thelohania* were known to occur in California mosquitoes. Nine species of *Thelohania* have now been described from the larvae of nine mosquito species in California and an additional eight infected mosquito hosts have been collected in either California or Nevada. The taxonomic placement of the *Thelohania* species from these new hosts awaits the ability of the researcher to infect mosquito hosts in the laboratory and demonstrate host specificity.

We were unable to influence the size and shape of *Thelohania californica* spores in a laboratory colony of *Culex tarsalis* by such physical factors as ranges of crowding, available food, and temperature or in field collected material from various elevations. Preservation of field collected infected material, without affecting the size and shape of the spores, was best attained by placing infected larvae in 10% formalin.

Only male larvae of some mosquito species exhibit frank infections whereas such infections develop in both male and female larvae of other mosquitoes. Transovarian transmission has been observed in several mosquito species.

Generally much less than 5% of any larval population in the field contains frank *Thelohania* infections.

Microsporidiosis of Larval Tabanidae

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Larvae of the black horse fly, *Tabanus atratus* Fabricius, collected in the Mississippi River Delta area near Greenville, Mississippi, were naturally infected with Microsporidia. In a single collection of 102 specimens, made in April 1963, 21 infected individuals were discovered. Examination of the parasite revealed a new species of the genus *Thelohania*. This parasite, which develops in muscle tissue, is highly pathogenic and is transmitted by ingestion of viable spores.

A second collection of 100 *T. atratus* larvae, made from the Greenville area in October of the same year, produced 4 individuals with microsporidian infections. Examination of the parasites in three of these larvae revealed (1) *Plistiphora* sp., (2) *Thelohania* sp. indistinguishable from the *Thelohania* sp. in the April collection, and (3) *Thelohania* sp. similar also to the parasites in the April collection but localized in the fat tissues. The latter microsporidian was transferred in the laboratory to healthy *T. atratus* larvae where, on the first passage, it again developed in fat tissues. However, on the second passage it developed exclusively in muscular tissue and became taxonomically indistinguishable from *Thelohania* sp. from the April collection.

The Importance of Taxonomic Investigations to Biological Control Operations

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Taxonomic investigations, or services, are of fundamental importance in other fields of entomological research, both basic and applied. This relationship is so close in the case of biological control that for some years the divisional investigations in these two areas have been placed together in the Insect Identification and Parasite Introduction Research Branch. The speaker will discuss the varied facets of this relationship such as the requirement for basic identifications, the role of the taxonomist in cataloguing, digesting and organizing knowledge about various taxa, and the role that the taxonomist can play in suggesting promising areas for field investigations.

The Analysis of Biological Control Effects Through Mathematical Models

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Truly efficient use of biotic agents for controlling destructive insects must be developed from a sound and thorough understanding of the basic population processes that determine the changes in numbers and qualities of each insect in question. In discussing or analyzing our attempts to manipulate either the extrinsic factors or processes and the intrinsic properties of a pest insect, we must distinguish between those that affect changes in numbers solely and those that affect population trends. Generally, the regulation or maintenance of an insect population at nondamaging levels by the latter mechanism is implied or stated as the aim of biological control. Yet it cannot be assumed that <u>all</u> biological agents have this effect.

Mathematical models are simply formalized statements (in the symbology of mathematics) of the relationships among some quantifiable entities. Such models may be explanatory, descriptive, or predictive. Thus, they may assist in unraveling biological complexities or serve simply to describe observed phenomena or predict outcomes and events.

The approach suggested here is that mathematical models be developed, tested, and used for (1) describing the basic population trend characteristics of the pest insect under natural conditions, (2) determining the age interval in the life history of the insect in which survival most strongly affects population trend, and the mortality-causing agents operating in that critical period that determine the survival rate therefrom, (3) comparing the effects of introduced agents, treatments, or combinations thereof, and deriving the "optimal treatment" for different objectives, (4) clarifying research objectives in biological control and orienting research programs most efficiently, and (5) integrating the biological, economic, and sociological factors for control decisions and long-term control policy.

The analysis and interpretations of mathematical models are only as good as the data entered into them. The models supplement, or make use of, biological knowledge; they do not replace it. And the data used must contain minimal sampling error.

Some examples illustrate these points.

Logical Implications of Population Theory

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If a population is considered to be a series of variable numbers, then the mean of plus values must equal minuses.

In most current models balance is obtained by assuming that the mean rate of increase equals 1. Balance will also result if limits exist. With this postulate, density-dependent controls are not necessary and the factors influencing birth and mortality can be independent of density and each other. It then becomes possible to construct empirical models of various levels of complexity. The simplest show that insect populations are affected by multiple variables that always include resources, weather, and enemies. If numerical values are assigned to these factors the resulting equations may become very complex. In theory it is possible to cope with any degree of complexity by solving the equations with computers, but in practice the limitations of measurement permit the inclusion of only a few variables in partly hypothetical model. The most realistic of these have been constructed by Morris and his co-workers. These models include measurements of a few direct factors and estimates of the effect of some indirect ones. At the present level of development they yield fairly accurate predictions of population variation in time but less information on factors causing variations in space or those that determine the mean level of abundance. Thus current models are of limited value for the purpose of estimating the reduction in populations to be expected from the introduction of additional mortality factors, such as natural enemies, into the ecosystem.

Morris

Insect Population Management Through Integrated Control

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Integrated control is the coordination of biological and chemical controls to reduce and maintain insect populations below economic thresholds.

Biological control has traditionally meant the action of parasites, predators, and pathogens on an insect population. Today it also includes nutritional, metabolic, and genetic controls, such as resistant varieties, sterilization, inheritable lethal genes, attractants, etc.

The ecological approach to insect control must be foremost. We must consider the ecosystem of each subject insect. In this way the aspects of our various researches can produce a complete picture. This approach might better be termed "economic insect ecology."

We must use preventive and "calendar" applications of pest control materials only as pest populations exceed economic thresholds. This means population prediction methods must be improved.

Our pesticide applications should become more and more selective either through the material applied or its method and timing.

We should consider regional eradication of particular pest species after research has developed the means. Integrated control can be utilized to reduce the pest population for eradication attempts to have the fullest chance to succeed.

Investigation Potentials in Bio-Control

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Exploration in the field of biological control has evolved from unpremeditated and unorganized beginnings in which accident was a dominant factor, to a highly systematized purposeful effort. A critical element in bio-control exploration, as with geographical exploration, is in keeping the "charts" or guide lines to future exploration, up to date.

Despite some remarkable accomplishments in the field of bio-control, exploration in this area is still in a very early stage.

No area of bio-control has been thoroughly explored and in some areas exploration has barely progressed beyond the nebulous stage of contemplation.

In addition to the area of attractants which we are learning how to exploit as a biological control weapon, is the need for more intensive study of the plant kingdom's own devices, evolved over the millions of years, for protecting its forms against harmful insects and pathogens. Examples of numerous other areas containing large exploration voids are the many families of parasitic and predatory insects, the life histories and habits of which are still largely unknown, the insect pathogens concerning which there is not only a dearth of knowledge but an equal dearth of trained personnel for its extraction, and techniques for rearing insects for biological control studies.

The study of living insects, necessary to an understanding of their biology, physiology, life history, and habits, has suffered proportionately from the amount of time traditionally devoted to the study of dead insects. The present research emphasis in the rearing of insects is a commendable trend which should serve in some measure to correct this inbalance and provide valuable data usable in the biological control of economic insect pests.

Reproductive Capacities as Indicators of the Effectiveness of Three Parasites of *Therioaphis maculata* in Various Climates

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The question is asked: Is it possible to measure the relative effectiveness of a parasite or parasites in various climates? To partially answer this question a laboratory study of the effect of several constant temperatures on an aphelinid and two braconid parasites of the spotted alfalfa aphid is discussed.

In the study, life tables were constructed for each parasite at each temperature, and various calculations from these life tables were obtained. There are certain problems in using parasites for this type of study.

Statistics such as developmental time, longevity, fecundity, generation time, reproductive rates, and innate capacity for increase, some of which are obtainable only from life table studies, are evaluated as to their relative advantages and limitations. These statistics are compared for each parasite species, and on this basis, an attempt is made to predict what might be expected of each parasite in the field.

Specificity in Host-Parasite Relationships with Particular Reference to Parasites of Hypera postica and their Relationship to Other Species of the Genus Hypera

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Parasites of Hypera postica (Gyllenhal), H. brunneipennis (Boheman), H. nigrirostris (Fabricius), and H. punctata (Fabricius) are discussed as to their host relationships and specificity within the genus. The relationships are as follows: (1) adapted hosts (those hosts in which oviposition and successful development occurs); (2) unadapted hosts (those hosts in which no oviposition occurs or if it did host defense reactions inhibit parasite development); and (3) partially immune hosts (those in which only a portion of parasitized hosts develop successfully). The greatest degree of specificity is found in those species which develop as solitary endoparasites of larvae. Specificity is usually determined by a host defense reaction commonly referred to as phagocytosis and results in the deactivation by encapsulation of parasite eggs and larvae by blood cells of the host.

Biological Control of Cephus in Eastern United States - a Success or a Failure

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Collyria calcitrator (Grav.) was introduced into Eastern United States from England in 1935-38 by the U.S.D.A. to control the European wheat stem sawfly, *Cephus pygmaeus*, and the black grain stem sawfly, *Cephus tabidus*. The project was regarded as a failure since there was no evidence that the parasite became established. The sawflies declined, however, and are of little economic importance today.

In 1957 *C. calcitrator* was discovered in New Jersey, and subsequent surveys have shown that it is widely distributed in Northeastern United States and destroys over half of the overwintering larvae of *C. pygmaeus*. The decline of *Cephus* after the release of *C. calcitrator* suggests that this is a highly successful case of biological control, but other evidence indicates that the parasite may not have been responsible for the decline of *Cephus*. A thorough study of the effect of *C. calcitrator* will be necessary to properly evaluate the importance of this parasite on the control of *Cephus*.

Strongly Density-dependent Factors versus Weakly Density-dependent Factors as Biological Control Agents

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The term "density-dependent" has been used by many ecologists, and many factors have been demonstrated to work in a density dependent manner at times. My original title, "Density-dependent versus Density-independent Factors as Biological Control Agents," is not accurate for the factors I had selected for comparison. Solomon (1949) had suggested that perhaps degrees of density-dependence might have to be considered. This is the procedure I have taken.

Strongly density-dependent factors are those which do not operate effectively until a high population of the host is present.

Weakly density-dependent factors can operate effectively under low host populations.

Strongly density-dependent factors discussed are chytrid of purple scale, Entomophthora of citrus red mite and a disease of citrus rust mites. Weakly density-dependent factors are Aphytis lepidosaphes, Compere, a parasite of purple scale and Aphytis holoxanthus DeBach, a parasite of Florida red scale. Data is presented which shows that the strongly host density-dependent factors take a higher proportion of the host population under high host population situations while the weakly host density-dependent factors are able to maintain a high degree of pressure when the host is at low density levels.

Parasites and Predators of Potato-Infesting Aphids in Maine

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In northeastern Maine, during 21 years of study, approximately 42 species of solitary, internal hymenopterous parasites were reared from the four potato-infesting species of aphids occurring there. These are the buckthorn aphid, Aphis nasturtii Kaltenbach, which ordinarily is by far the most abundant species but the least parasitized, the green peach aphid, Myzus persicae (Sulzer), intermediate in abundance, and the foxglove aphid, Acyrthosiphon solani (Kaltenbach), least abundant of the four species, are subject to only slightly greater parasitism; and the potato aphid, Macrosiphum euphorbiae (Thomas), of intermediate abundance, is the most heavily parasitized. Of the 24 species of primary parasites, ordinarily only a few are common; the one by far most abundant is Aphidius nigripes Ashmead, followed by Praon pequodorum Viereck and two underscribed species of Praon. Only a few of the 18 species of hyperparasites are very abundant, including Asaphes lucens (Provancher), 3 species of Lygocerus, and Coruna clavata Walker.

In the potato aphid on potatoes, during the period 1952 through 1962, total-season hyperparasitization has averaged 22 percent, varying yearly from 13 to 45 percent. By the criteria used, hyperparasitism appears to have had no appreciable or consistently deleterious influence upon population sizes or trends or parasitism of the potato aphid; neither has the parasitism encountered had an appreciable or consistently adverse effect upon rate of population increase of this aphid.

Arthropod predators of these aphids have included chiefly spiders, coccinellids, syrphids, and chrysopids. In two years and possibly a third one out of 12, predators have been abundant and effective enough to be a potent factor in control of aphids on potatoes not treated with insecticides.

Contrasted with insect parasites and predators, parasitic fungi each year have exerted a very substantial degree of control of the potato aphid; however, it has varied considerably from year to year because of differences in starting time, intensity, and duration of activity of the fungi.

The information and experience gained in our study appears to justify initiation of intensive efforts to enhance control of aphids on potatoes with biological agents, including insect parasites, predators, and entomogenous fungi, particularly the latter. Likelihood of success with insect parasites and predators will depend upon their uniform distribution in potato fields in large enough numbers to create a catastrophic pressure of short duration soon after completion of the aphids' spring migrations each year. Success with entomogenous fungi will depend upon both distribution and germination of the spores early in the growth phase of the curve of seasonal population trend of the aphids.

Methods Used in Propagation, Colonization, and Recovery of Some Introduced Parasites of the Alfalfa Weevil

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Three introduced parasites of the alfalfa weevil are established and thriving in parts of Eastern United States. Some important factors that affect propagation in the laboratory are discussed. The importance of the selection of favorable sites for parasite release is stressed particularly with regard to the colonization of *Microctonus aethiops*. Mention is made of the procedures used in recovery and the determination of parasite establishment.

Introduction of Agathis pumils Against Larch Casebearer in Idaho

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The larch casebearer, Coleophora laricella (Hbn.), was discovered in 1957 at St. Maries, Idaho. The severity of defoliation at this time showed that the species had been present for some time before discovery. The rate of spread of the casebearer has been phenomenal. From 1957 to 1959 defoliation was visible over an area of 170 square miles. In 1963 larch type in an area of 2,000 square miles was noticeably defoliated and the limits of dispersal covered an area of 8,000 square miles. The Braconid parasite, Agathis pumils, was introduced into Idaho in 1960 in the adult stage. The parasites were reared from material collected in New England and shipped to Idaho. The parasite became established and adults have been recovered in 1962 and 1963, which offers encouragement in this program of biological control.

[illegible]

Biological Control of Forest Insects in the Southeastern Region With Special Emphasis on the Balsam Woolly Aphid

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In general, research in biological control of forest insects in the Southeast has been empirical and conducted by few agencies. Most intensive has been the study of native and imported predators for the control of the balsam woolly aphid.

Entomologists of the Southeastern Forest Experiment Station have conducted laboratory and field studies against pine coneworms, *Dioryctria* spp., with *Beauveria bassiana* and *Bacillus thuringiensis*; pine tip moths, *Rhyacionia* spp., with *B. t.*; red-headed pine sawfly, *Neodiprion lecontei* with polyhedral viruses; and elm spanworm, *Ennomos subsignarius*, with *B. t.* and a number of polyhedral viruses. With some exceptions, results have been promising in the laboratory but fair to poor in the field. The Virginia Division of Forestry had variable success with polyhedral viruses against *Neodiprion pratti pratti* in field studies, no success with *B. t.*

Parasites and predators have been studied little in control investigations in the Southeast. During 1959-1961 the Virginia Division of Forestry made substantial releases of the European cocoon parasite, *Dahlbominus fuscipennis*, against *N. pratti pratti* to supplement established populations. Its effectiveness appeared limited.

In North Carolina the Southeastern Forest Experiment Station has been employing introduced predators since 1959 in studies to control the destructive balsam woolly aphid, *Chermes piceae*. The European predators show most promise, especially *Laricobius erichsonii* Rosenh. and *Aphidecta obliterata* (L.). In 1962 over 12,000 *Laricobius* were released in the vicinity of Mt. Mitchell in a pilot test. In 1963 *Laricobius* was recovered at 7 of the 13 release sites. *Aphidecta* released in 1960 in small numbers, was being recovered in 1963. Predatory species introduced from Pakistan and India to date have shown little tendency to feed on the balsam woolly aphid or promise of establishment.

Studies of Diapause in Several Species of Parasitic Insects

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Laboratory studies are being conducted to determine the influence of photoperiod, temperature, female age, and periods of host-denial on the induction of diapause in three species of indigenous parasites, *Coeloides brunneri* Vier., *Itoplectis 4-cingulatus* (Prov.), and *Apechthis ontario* (Cress.). Some studies are aimed at determining the physiological mechanisms involved. Techniques being used are described. The possible applications of these studies and of laboratory studies of entomophagous insects in general are described in relation to the overall biological control effort.

Current Domestic Research in Biological Control of Weeds

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Since the success of the biological control program on Klamath weed, *Hypericum perforatum* L., primarily by the defoliating beetle, *Chrysolina quadrigemina* (Suffrian), a number of other weedy plant species have become the subjects for biological control research projects.

Present research projects, in addition to Klamath weed which is now only an occasional pest, include the following insect introductions on four weedy plants: a seed weevil, *Microlarinus lareynii* (Duv.), and a stem weevil, *M. lypriformis* (Woll.) on puncture vine, *Tribulus terrestris* L.; a twig mining moth, *Leucoptera spartifoliella* Hub., and a seed weevil, *Apion fuscirostre* F., on Scotch broom, *Cytisus scoparius* (L.); a seed weevil, *Apion ulicis* Forst., on gorse, *Ulex europaeus* L.; and a defoliating moth, *Tyria jacobaeae* (L.) on tansy ragwort, *Senecio jacobaea* L.

These insects are all comparatively recent introductions, with *A. fuscirostre* having been first released March 4, 1964. Although widespread control is not yet evident, these species, with the exception of *A. fuscirostre*, are all well established in a number of areas in the western United States.

Plans for 1964 call for the introduction of a European fleabeetle, *Altica carduorum* Guer., obtained through Canadian cooperators, for release on Canada thistle, *Cirsium arvense* Scop., and *Agasicles* n. sp., a fleabeetle from Argentina, for release on alligator weed, *Alternanthera philoxeroides* (Mart.) Griseb. In addition to these releases, a moth, *Heterographis fulvobasella* Ragonot, will be introduced into quarantine at Albany, California, for final testing work in anticipation of a 1965 release on halogeton, *Halogeton glomeratus* Mey., a poisonous plant of western rangelands.

Mechanisms of Host Acceptance in Weed Feeding Insects

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Data relative to the mechanisms of host plant selection by weed feeding insects are conspicuous because of their scarcity, although there is a growing interest in this phase of research. Much more is known about host selection in agricultural (crop feeding) insects. Several schools of thought differ slightly on what is considered the most important factor involving host acceptance.

The process of locating host plants by insects, and the follow-up mechanism of accepting the host for feeding or oviposition, are discussed. Such factors as mechanical and nutritional qualities of the plant, and characteristic non-nutritional chemicals associated with a plant or plant family are implicated. Often there appears to be a complicated interaction of several factors that suggests the plant and the insect have evolved very intimately.

The interest in this field is intense with those of us working in biological control of weeds, since the host specificity of each candidate insect must be known before it can be released into a new area for possible control of a weed.

Biological Control of Aquatic Weeds

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Our first attempt to suppress or control an aquatic weed, through deliberate introduction of a plant-feeding insect, is concerned with alligatorweed in the southeastern United States. After exhaustive surveys for insects associated with the weed in regions of South America to which alligatorweed is native, research to determine host specificity of the most promising insects was initiated. A flea beetle of the genus Agasicles has now been proven to feed only on alligatorweed. Larvae starved when offered crop plants or plants that were of wildlife or ornamental value. It is thus considered completely safe to release the flea beetle in the United States.

Two difficulties have arisen in carrying through our proposed introduction and release of this insect. The first problem is that of securing permission from regulatory officials in the Southeastern States for the introduction, a necessity before Plant Quarantine Division, ARS will allow entry of the insect. The second problem arises from the rapidity in development of the insect. Since there is no period in the insect's cycle when it diapauses, and since air transport time between Buenos Aires and the United States is unreasonably long, it is difficult to obtain living material. We hope solutions to both problems will soon be forthcoming.

Research has been initiated on a second insect, a thrips, to prove its specificity.

P.L. 480 Projects and Their Value to Biological Control Research in the United States

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P. L. 480 funds have been made available for a considerable number of entomological research projects since the foreign agricultural research grant program was begun in 1958. A number of them are directly concerned and many at least somewhat concerned with biological control problems. Current projects and accomplishments are summarized briefly. Methods for submitting proposals are discussed. Consideration is given to the value of projects now underway and the potential value of future projects.